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The active heroin metabolite 6-acetylmorphine has powerful reinforcing effects as assessed by self-administration in the rat

Riccardo Avvisati*[1], Inger Lise Bogen[2,3], Jannike M. Andersen[2,4], Vigdis Vindenes[2,5], Jørg Mørland[5,6], Aldo Badiani[1,7], Fernando Boix[2].

[1]Sussex Addiction Research and Intervention Centre (SARIC), School of Psychology, University of Sussex, Brighton, UK; [2]Section of Drug Abuse Research, Department of Forensic Sciences, Oslo University Hospital, Norway; [3]Institute of Basic Medical Sciences, Faculty of Medicine, University of Oslo, Norway; [4]Department of Pharmaceutical Biosciences, School of Pharmacy, Faculty of Mathematics and Natural Sciences, University of Oslo, Norway; [5]Institute of Clinical Medicine, Faculty of Medicine, University of Oslo, Norway; [6]Norwegian Institute of Public Health, Oslo, Norway, [7]Department of Physiology and Pharmacology, Sapienza University of Rome, Rome, Italy

*Current address: MRC Brain Network Dynamics Unit, University of Oxford, Oxford, UK

Corresponding Authors:

Aldo Badiani: SARIC, University of Sussex, Sussex House, Falmer, Brighton, BN1 9RH, UK. E-mail: Aldo.Badiani@sussex.ac.uk

Fernando Boix: Section for Drug Abuse Research, Dept. of Forensic Sciences, Oslo University Hospital, PO Box 4950 Nydalen, N-0424 Oslo, Norway. E-mail: fernando.boix.escolan@ous-hf.no

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ABSTRACT

Previous studies have suggested that at least some of the behavioral effects of heroin might be mediated by its active metabolite 6-acetylmorphine (6-AM). The aim of the present study was to investigate the reinforcing effects of 6-AM and its role in mediating those of heroin. We used an intravenous self-administration procedure in male Sprague-Dawley rats including four phases: acquisition, extinction, relapse into drug-seeking, and re-acquisition. Animals learned to self-administer 0.135 µmol/kg (44.3 µg/kg) 6-AM at similar rates as an equimolar dose (50 µg/kg) heroin under fixed ratio 1 (FR1), but a significantly higher rates under FR2. No differences were observed during extinction. Priming with 0.068 µmol/kg reinstated lever presses for both drugs during the first hour of the relapse session. Re-acquisition of self-administration with the dose used during acquisition (0.135 µmol/kg) restored lever presses for both drugs at a level similar to the last acquisition session. However, re-acquisition using half the dose (0.068 µmol/kg) significantly increased self-administration in animals receiving 6-AM, but not in animals receiving heroin. Administration of a specific monoclonal antibody against 6-AM before the relapse session blocked relapse of self-administration of 6-AM and affected later re-acquisition in a manner compatible with a shift to the right of the dose-effect curve. Only minor effects of the antibody were observed on the reinstatement of heroin-seeking (relapse) and on the re-acquisition of heroin self-administration. The present results show that intravenous 6-AM possesses similar reinforcing properties as heroin. However, some observed disparities also indicate some differences in their rewarding qualities after systemic administration. The lack of effect of the 6-AM antibody over relapse and re- acquisition of heroin self-administration calls for further studies to clarify the role of heroin and its metabolites in heroin reward.

Keywords:

Heroin, 6-acetylmorphine, self-administration, reinforcement.

Abbreviations:

6-AM: 6-Acetylmorphine

1. INTRODUCTION

Heroin (diacetylmorphine) is an acetylated derivative of morphine with a high addiction potential, higher than for any other substance, except tobacco (Darke, 2011; Nutt et al., 2007). The past few years in particular have seen a sharp increase in heroin abuse and, as a consequence, an alarming rise in the number of heroin-related overdoses and deaths (Dowell et al., 2017; European Monitoring Centre for Drugs and Drug Addiction, 2018). Yet, despite decades of research on its pharmacodynamics and pharmacokinetics, the mechanisms responsible for the reinforcing effects of heroin, the basis of its high abuse potential, are still poorly understood.

Heroin's estimated plasma half-life is less than 5 minutes, being rapidly transformed into 6-acetylmorphine (6-AM), which is further de-acetylated to morphine, both in rodents (Andersen et al., 2009; Gottas et al., 2013) and in humans (Rook et al., 2006a; Rook et al., 2006b). Heroin, 6-AM, and morphine are all μ-opioid (MOP) receptor agonists (Inturrisi et al. 1983, Selley et al. 2001). The very fast metabolism of heroin, and their very similar pharmacological profiles, led to hypothesize that the effects of heroin are mostly mediated by morphine (Way et al., 1960; Wright, 1941). However, there is some evidence against this simplification. The euphorigenic effect of heroin (the so-called 'rush' or 'high') develops very rapidly after systemic administration (Smith and Beecher, 1962), whereas morphine concentrations in the plasma increase much more slowly (Rook et al., 2006a; Rook et al., 2006b), and, owing to its low lipophilicity, even more slowly in the brain, (Gottas et al., 2014; Seleman et al., 2014). Thus, the attention of researchers has shifted to 6-AM, which binds MOP receptors with the same affinity of morphine (Inturrisi et al., 1983) but has greater efficacy (Selley et al., 2001). In humans, 6-AM is found at higher concentrations than morphine immediately after heroin administration, overlapping in time with heroin's 'high' (Rook et al., 2006a; Rook et al., 2006b). In rodents as well, 6-AM represents the main metabolites for the first 30 min after heroin administration (Andersen et al., 2009; Gottas et al., 2014; Gottas et al., 2013). Thus, 6-AM is a clear candidate to convey the reinforcing properties of systemic heroin.

It has been shown that systemic administration of 6-AM can produce analgesia and constipation (Umans and Inturrisi, 1981), as well as psychomotor activation (Andersen et al., 2009) similar to that produced by heroin. Interestingly, the time-course of striatal concentration of 6-AM parallel those of locomotor activity (Andersen et al., 2009) and striatal dopamine levels (Gottas et al., 2014) after heroin administration. Still, relatively little is known about the rewarding properties of 6-AM. Thus, the main aim of the present study was to compare the reinforcing effects of equimolar doses of 6-AM and heroin using an intravenous (i.v.) self-administration procedure in the rat. The second goal was to assess the effects of a pretreatment with a specific monoclonal antibody (mAb) raised against 6-AM (Bogen et al., 2014; Kvello et al., 2016) on the reinforcing effects of heroin, and in particular on its ability to affect reinstatement of drug-seeking in animal model of relapse (for a review, see Shaham et al. 2003) and to sustain self-administration.

2. MATERIAL AND METHODS

2.1 Animals

A total of 89 male Sprague-Dawley rats (ENVIGO, Huntingdon, United Kingdom) weighting 250-275 gr at the beginning of the experiment were used in this study. Rats were housed in a temperature- and

humidity-controlled room (21 ±1° C and 55 ± 5%, respectively) on a 12/12 h reverse light cycle (lights off at 8:00 am) for the entire duration of the experiments. Upon arrival, rats were housed in triplets in grid-top plastic cages (52 cm length, 40 cm width and 27 cm height) with wood shavings bedding and brown paper nesting material and allowed to acclimatize to the animal facility for a week. After this period, the animals underwent i.v. catheterization surgery. Eleven to sixteen animals were tested simultaneously, representing the different treatment groups, during one entire self-administration procedure lasting 17 days, with one session per day. The rats had *ad libitum* access to food and water throughout the experiment, except during the self-administration sessions. All experimental procedures on animals were conducted in accordance with UK Animals (Scientific Procedures) Act 1986 (www.legislation.gov.uk/ukpga/1986/14/contents).

2.2 Surgery

On the day of surgery, the rats received an intraperitoneal injection of 2mg/kg of xylazine hydrochloride (Rompun, Bayer HealthCare) and 100mg/kg of ketamine (Anesketin, Dechra). The surgical procedures were similar to those previously described by (Caprioli et al., 2008). Briefly, an 11 cm silicone catheter (0.37-mm inner diameter and 0.94-mm outer diameter), sheathed at 3.4 cm from its proximal end by a silicone bead, was inserted into the right jugular vein and secured to the surrounding soft tissues with silk thread. Its distal end was externalized through a small incision at the nape of the neck and connected to an L-shaped 22-gauge cannula, which was secured to rat's skull using dental cement and stainless steel screws. The catheters were flushed daily (at 6 pm) with 0.1 ml of sterile saline. Immediately after surgery, rats were assigned to the heroin or 6-AM group and individually housed in the self-administration chamber, where they remained for the rest of the experiment. The rats were allowed to recover from the surgery for 7–10 days before starting self-administration training.

2.3 Apparatus

The apparatus, previously described in detail by Caprioli et al. (2007), consisted of self-administration chambers (28.5-cm length, 27-cm width and 32-cm height) made of transparent plastic (front and rear walls), aluminium (sidewalls and ceiling) and stainless steel (grid floor). Plastic trays covered with pinewood shaving were placed under the cage floors. Each cage was equipped with a counterbalanced arm holding a liquid swivel and two retractable levers, positioned 12.5 cm apart and 9 cm above the floor on the left-hand wall. Cue lights consisting of either a set of triple (green, red and yellow) LED lights were positioned above each of two levers. The self-administration cages were placed within sound-attenuating and light-attenuating cubicles. Each cage was connected via an electronic interface to a syringe pump (Razel Scientific Instruments, St. Albans, VT, USA) and to a programmable logic controller (PLC; Allen Bradley, Milwaukee, WI, USA). Finally, the PLCs were connected to PCs running custom control software developed by Aries Sistemi S.r.l. (Rome, Italy).

2.4 Drugs

Heroin hydrochloride (Johnson Matthey-Macfarlan Smith, Edinburgh, UK) and 6-AM hydrochloride (Lipomed, Arlesheim, Switzerland) were dissolved in saline. Equimolar doses of heroin and 6-AM, an infusion volume of 40 µl, and an infusion time of 4 s, were used throughout the experiment. During the acquisition phase (see Section 2.5.1), a unit dose of 0.135 µmol/kg (corresponding to 50 µg/kg of heroin and to 44.3 µg/kg of 6-AM) was used. These doses were selected on the basis of previous studies (Caprioli et al. 2008; Montanari et al. 2015). The priming doses for the relapse session (see

Section 2.5.3) were 0.017, 0.034, or 0.068 μmol/kg (2.5, 12.5 or 25 μg/kg for heroin and 2.21, 11.1 or 22.2 μg/kg for 6-AM, respectively). The priming doses were selected based on a study showing that 25 μg/kg of heroin was a more effective priming dose than 50 or 100 μg/kg for inducing relapse into heroin-seeking (Montanari et al. 2015). Two doses, 0.068 or 0.135 μmol/kg (equivalent to 25 and 50 μg/kg for heroin, and 22.15 and 44.3 μg/kg for 6-AM, respectively), were used for the re-instatement sessions (see Section 2.5.4).

2.5 Self-administration procedure

Figure 1 illustrates the timeline of the experimental protocol.

Prior to the beginning of each session food, water and nesting material were removed from the self-administration chamber and infusion lines were sterilized with ethanol, flushed with sterile saline, and then loaded with the appropriate drug solutions. At the beginning of each session, the two levers were extended (one 'active' and one 'inactive', counterbalanced for the right vs. left position) and the cue light (all three LEDs) above the active lever was switched on. The schedule requirement, the number of consecutive lever presses, to obtain an infusion was increased from fixed ratio 1 (FR1) during the first 5 sessions, to FR2 for the rest of the experiment. Each animal received the same drug, 6-AM or heroin, through all the experiment. All sessions lasted 6 hours, and the animals were housed in the self-administrations chambers for all the length of the experiment.

2.5.1 Acquisition

The rats were trained to self-administer 0.135 μmol/kg 6-AM or heroin during 7 consecutive daily sessions. After pressing the active lever at the programmed schedule, a drug infusion was delivered, the levers retracted, and the cue light switched off for a 40-s timeout period (to prevent self-administration of multiple consecutive infusions and overdosing). The rats that did not spontaneously self-administer at least one infusion within the first 5 min of the session were placed with their forepaws on the lever to prime an infusion. This was repeated at times 60 and 120 min for rats that did not self-administer at least one infusion in the time periods 5–60 and 60–120 min. These priming infusions were not included in the data analysis

2.5.2 Extinction

The extinction phase consisted of 7 consecutive daily sessions (sessions 8 to 14), during which the rats received, upon completion of the task (FR2), an i.v. saline infusion instead of the drug solution. All other conditions during the sessions were kept identical. No priming infusions were given during extinction.

At the end of session 14, the rats were administered either an intraperitoneal injection of 10 mg/kg of a human mAb raised against 6-AM (Bogen et al., 2014; Moghaddam et al., 2003) or an equivalent volume of saline and left undisturbed in their cages.

2.5.3 Re-instatement of drug-seeking (relapse session)

Before the beginning of the session (session 15), the infusion lines were backfilled with 50 μl of sterile saline containing one of the priming doses (0.017, 0.034, or 0.068 μmol/kg) of 6-AM or heroin. The rest of the infusion lines were filled with sterile saline. At the beginning of the session, a 10 -s, 100-μl infusion (consisting of 50 μl of the reinstatement dose followed by 50 μl saline solution) was

triggered. During the rest of the session, lever pressing of the active lever resulted in the self-administration of saline, as during the extinction sessions.

2.5.4 Re-acquisition of self-administration

During sessions 16 and 17 the rats were returned to a schedule of self-administration identical to that of sessions 6 and 7 (i.e., on an FR2 schedule of reinforcement), with the difference that on one session they received the dose used during training (0.135 µmol/kg) and on the other one half this dose (0.068 µmol/kg), in a counterbalanced fashion.

2.6 Quantification of IgG1.

At the end of session 17, the rats were sacrificed with an i.v. infusion of pentobarbital (4 mg in 20 µl), which also served to assess catheter patency. The animals that did not become ataxic and died within 5 seconds (a total of 7 rats) were excluded from the data analysis. After cervical dislocation and death confirmation, blood samples were taken by cardiac puncture with a heparinized syringe and put in 0.5 ml low protein binding Eppendorf tubes held in ice. The tubes were then stored in a -80°C freezer and later shipped to Oslo University Hospital (Oslo, Norway) for quantification of blood IgG1 levels.

Blood samples were diluted and analyzed for human IgG1 using a Novex ELISA kit (Thermo Fisher Scientific Inc., Waltham, MA). Two duplicates of each standard and control were used. Absorbance was measured (450 nm) within 1 hour of adding the stop solution using an ELx808 Absorbance Microplate Reader (BioTek Instruments Inc., Winooski, VT). All animals receiving the 6-AM mAb were tested, but only 11 animals pretreated with saline was analysed as control.

2.7 Data analysis

Self-administration and extinction data were analysed using a two-way mixed ANOVA for the between-subject factor *drug* (two levels: heroin vs. 6-AM) and the within-subject factor *session* (one level for each session). Sessions 1-5 (on FR1) and 6-7 (on FR2) were analysed separately. Relapse was defined as a significant increase on the number of presses on the active lever and/or in the number of infusions during the first hour of session 15 relative to the first hour of the last extinction session (session 14). Relapse data were analyzed using a three-way mixed ANOVA for the between-subject factors *drug* (two levels: heroin vs. 6-AM) and *antibody* (saline vs. mAb) and the between-subject factor *relapse* (two levels: session 14 vs. session 15). Re-acquisition of self-administration was indicated by a significant increase in the number of presses on the active lever and/or in the number of infusions during session 16 or 17 relative to the last extinction session (session 14). Re-acquisition data were analysed using a four-way mixed ANOVA for the between-subject factors *drug* (two levels: heroin vs. 6-AM), dose (two levels: 0.068 vs 0.135 µmol/kg) and *antibody* (saline vs. mAb) and the between-subject factor *re-acquisition* (two levels: session 14 vs. session 16 or 17). Student's t-tests for independent or repeated measures were used, as required, for post-hoc comparisons of interest. The statistical analysis was run separately for active lever presses, inactive lever presses, and drug infusions. All statistical analyses were conducted using SPSS statistical software version 23 (IBM Corp, Armonk, NY, USA).

3. RESULTS

3.1 Acquisition (Figure 2)

During sessions 1-5, animals learned to self-administer 6-AM or heroin (on FR1), as indicated by a significant increase in lever presses over session (Session: $F_{4,320}$=43.98, p<0.0001), with no significant effect of drugs (Session x Drug: $F_{4,320}$=1.94, p=0.104; Drug: $F_{1,80}$=2.83, p=0.096). Also for sessions 6-7 (on FR2) there was a significant effect of session ($F_{1,80}$=138.53, p<0.0001), but in this case also a significant effect of drug ($F_{1,80}$=4.70, p=0.033) and a drug x session interaction ($F_{1,80}$=4.53, p=0.036), as lever pressing for 6-AM was greater than for heroin. The same pattern was observed for the number of infusions.

3.2 Extinction (Figure 2)

During the extinction sessions, there was a significant decrease in the number of presses on the active lever ($F_{6,480}$=17.87, p≤0.0001) with no main effect of drug ($F_{1,80}$=0.39, p=0.843) nor session x drug interaction ($F_{6,480}$=0.779, p=0.527). The same pattern was observed for the number of saline infusions.

3.3 Relapse into drug-seeking (Figure 3)

Since priming with 0.017 or 0.034 µmol/kg 6-AM or heroin did not induce relapse (data not shown), the use of these doses was stopped and only the dose of 0.068 µmol/kg was used for priming during the rest of the experiment. Due to the low number of animals for these doses (total n=16 and 21 for each dose, respectively) within each level of the treatment factors (heroin vs 6-AM and saline vs mAb), no statistical analysis was carried out.

Priming with 0.068 µmol/kg reinstated lever pressing on the drug-paired lever (Relapse: $F_{1,41}$=18.11, p=0.000) and the number of infusions (Relapse: $F_{1,41}$=17.70, p=0.000) during the first hour after priming, without affecting presses at the inactive lever (data not shown). The post-hoc tests showed that priming with both drugs reestablished self-administration in the saline pretreated animals, but in the animals pretreated with the 6-AM mAb only the group receiving heroin showed relapse.

3.4 Re-acquisition of self-administration (Figure 4)

When the drugs were made available for the re-acquisition of self-administration on sessions 16 and 17, there was a significant effect in the number of active lever presses ($F_{1,74}$=89,52, p<0.0001) and in the number of infusions ($F_{1,74}$=85,05, p<0.0001) relative to last extinction session (session 14). This effect was depending on which drug animals received, which dose, as well as pretreatment with the 6-AM antibody, both for the number of presses at the active lever (re-acquisition x drug x dose x pretreatment : $F_{1,74}$=9,45, p=0.003) and number of infusions (re-acquisition x drug x dose x pretreatment : $F_{1,74}$=8,08, p=0.004).

Post-hoc analysis showed that the rats pre-treated with saline resumed lever pressing on the active lever and received a number of infusions significantly greater than on the last extinction session and at least similar to those of the last session of acquisition (session 7), independently of the drug or dose received. However, the animals receiving 0.068 µmol/kg 6-AM exhibited even a nearly statistical significant (p=0.065) higher number of presses on the active lever and a significant (p<0.05) higher number of infusions than observed on the last acquisition session.

In the animals pretreated with the 6-AM mAb, the effects differed depending on the drug and dose received. In those animals receiving 0.068 µmol/kg 6-AM and pretreated with the 6-AM mAb, the number of active lever presses and infusions received were not significant different either from the

performance on the last acquisition day or from the last day of extinction. On the contrary, the animals pretreated with the mAb and receiving 0.135 µmol/kg 6-AM showed a significant ($p<0.05$) increase in the number of presses at the active lever and the number of infusions, not only compared against saline pretreatment, but also compared against the performance observed during the last day of acquisition. Pretreatment with the 6-AM mAb did not affect the re-acquisition of self-administration of heroin compared with the saline pretreated animals. However, the number of infusions were statistically significant higher ($p<0.05$) compared to the last acquisition day in the animals receiving 0.135 µmol/kg heroin, despite the effect size being small. There were not observed any significant effect on the number of presses on the inactive lever during the re-acquisition phase. No significant differences between saline and 6-AM mAb pretreatment were observed during the second day of re-acquisition for any of the two drugs and doses used (data not shown).

3.5 IgG levels

As expected, IgG levels were significantly ($p<0.001$) higher in animals pretreated with 6-AM mAb (35.2 ±2.8 µg/ml) compared with animals pretreated with saline (0.18 ±0.09 µg/ml). No differences were observed in IgG levels between animals self-administering 6-AM or heroin (33.3 ±3.5 µg/ml and 35.2 ±2.8 µg/ml, respectively).

4. DISCUSSION

The present study shows that 6-AM has powerful reinforcing effects, as indicated by its ability to sustain i.v. self-administration in the rat. The rats that had acquired 6-AM self-administration also exhibited relapse into drug-seeking when primed with a single infusion after a period of abstinence and then rapidly resumed self-administration behavior when given access to 6-AM again. This is, to the best of our knowledge, the first report to demonstrate the reinforcing effects of 6-AM in a self-administration procedure, considered the standard animal model for drug addiction (Spanagel, 2017).

4.1 Reinforcing effects of 6-AM vs. heroin

The reinforcing effects of 6-AM were similar to those of heroin, including the ability to trigger relapse into drug-seeking after a period of abstinence. However, the patterns of self-administration for 6-AM presented interesting differences from that of heroin. Although during the acquisition phase there were not statistical differences in the rate of responding on FR1, there was a significantly higher rate and greater number of infusions for 6-AM versus heroin on FR2. Furthermore, during the re-acquisition phase, the response rate and number of infusions for rats self-administering 0.068 µmol/kg of 6-AM was nearly twice as high as that of rats self-administering 0.135 µmol/kg (the dose used during the acquisition phase). On the contrary, the response rate and number of infusions for the two heroin doses did not differ from each other. These data seem to suggest that when administered systemically 6-AM is not only more potent than heroin with regard to its reinforcing effects but it also has greater efficacy.

4.2. Role of 6-AM in the reinforcing effects of heroin

The ability of 6-AM to sustain self-administration is consistent with the notion that 6-AM might represent the main mediator of the reinforcing effects of heroin. Raleigh and colleagues (Raleigh et

al., 2014), for example, in reporting that a morphine-conjugated vaccine can alter heroin self-administration in the rat, attributed this effect mainly to a reduction in brain levels of 6-AM. Thus, we hypothesized that a pre-treatment with specific mAb against 6-AM would block, or at least impair, heroin self-administration. Yet, we found that while mAb was effective in blocking 6-AM-induced relapse of drug seeking and altered the re-acquisition of 6-AM self-administration (in a way compatible with a shift to the right in the dose-effect curve), it had a negligible effect on heroin-induced relapse and on the re-acquisition of heroin self-administration, even though at the end of the experiment there were no differences in IgG levels between 6-AM and heroin groups.

This finding does not appear to be consistent with the hypothesized role of 6-AM in the reinforcing effects of heroin. In a preliminary experiment conducted in preparation for the present study (data not shown), an intraperitoneal injection of 7.5 mg/kg of mAb 15 min before an i.v. infusion of 0.135 µmol/kg of heroin reduced brain levels of 6-AM by 30-50% (measured 5 min after heroin administration). Previous experiments have shown that, after heroin administration in mice, this mAb against 6-AM is able to reduce 6-AM levels in the brain, accompanied by decreases in the heroin-induced psychomotor activity (Bogen et al., 2014; Kvello et al., 2016). In a recent study, this mAb was able to block the conditioned place preference (CPP) induced by heroin in mice (Kvello et al., Submitted), indicating that blockade of 6-AM is able to affect at least some aspects of the rewarding properties of heroin. Thus, the lack of effect of the mAb on heroin self-administration cannot be attributed to a possible general lack of action on 6-AM generated by heroin.

There are possible reasons why anti-6-AM mAb failed to affect heroin self-administration and heroin priming. The first one concerns the kinetics of heroin deacetylation. Most of the 6-AM found in the brain shortly after heroin administration originates from heroin deacetylation in the blood (Boix et al., 2013). Still, 6-AM levels in the brain are much higher and rise faster after administration of heroin than after administration of equimolar doses of 6-AM (Andersen et al., 2009; Gottas et al., 2014). This 'additional' amount of 6-AM most likely derives from the deacetylation of heroin in the brain after its rapid passage through the blood-brain barrier (Oldendorf et al., 1972) and is obviously out of the reach of the mAb. Indeed, brain 6-AM levels in the brain are reduced by mAb to a lesser extent after heroin administration than after 6-AM administration (Kvello et al., 2016). The mAb is also much less effective in reducing heroin-induced behavior immediately after heroin administration than later in time (i.e figure 3 in (Bogen et al., 2014)). Thus, the rapid intracerebral formation of 6-AM from heroin could be fast enough to sustain self-administration at the infusion dose used (Comer et al., 1999; Marsch et al., 2001; Samaha and Robinson, 2005) and would explain the lack of the effect of the mAb. However, it would be then necessary to assume that the 6-AM formed in the brain is sufficient to drive heroin self-administration but not heroin-induced CPP or locomotor activity. This discrepancy is not entirely surprising, given that he underpinnings of self-administration, locomotor activity, and CPP have been shown to be largely independent (e.g., Bardo and Bevins 2000; Ahmed and Cador 2006; Dietz et al. 2007; Shabat-Simon et al. 2008; Caprioli et al. 2008; Huston et al. 2013). In this respects, it is important to point out that although the mAb used here has very low affinity for heroin (Bogen et al., 2014; Kvello et al., 2016) a higher dose of the mAb (Kvello et al., Submitted) might have been effective.

Alternatively, it is possible that the reinforcing effect sustaining heroin self-administration is mediated by other active metabolites of heroin. Heroin has been considered to be only an effective vehicle to deliver morphine to the brain. However, the euphorigenic effect of heroin (the so-called 'rush' or 'high') develops very rapidly after systemic administration (Smith and Beecher, 1962),

whereas morphine concentrations in the plasma increase much more slowly (Rook et al., 2006a; Rook et al., 2006b), and, owing to its low lipophilicity, even more slowly in the brain, (Gottas et al., 2014; Seleman et al., 2014). This explains why higher doses of morphine than of heroin, are necessary to produce comparable behavioral responses (Andersen et al., 2009; Bardo et al., 1995; Eriksen et al., 2014; Hubner and Kornetsky, 1992; Hutto and Crowder, 1997), and in particular to sustain self-administration (Walker et al., 1999). Besides, the levels of morphine reached in the brain after systemic heroin administration are not enough to achieve an equivalent behavioral response as the one induced by heroin (Andersen et al., 2009). Another candidate for mediating the effects of heroin is morphine-6-glucuronide (M6G) (Vindenes et al., 2006; Vindenes et al., 2008). Although very little M6G is formed after heroin administration to drug-naïve rats, increased synthesis is observed after repeated exposure to heroin (Antonilli et al., 2003, 2005) as well as after heroin-self-administration (Meringolo et al., 2012). Yet, the time-course of M6G formation is even more delayed than that of morphine, making it less likely that it could significantly contribute to the rapid onset of heroin rewarding effects.

Finally, it is possible that the pharmacodynamics properties of heroin cannot be reduced to that of its metabolites. While heroin has lower affinity for MOP receptors than 6-AM or morphine (Inturrisi et al., 1983), its efficacy is similar to 6-AM and greater than morphine (Selley et al., 2001). Heroin and its metabolites differ not only in terms of receptor affinity and efficacy but also in their ability to enhance dopamine release in the striatum. Indeed, Gottas and colleagues (2014) have shown that the temporal and quantitative pattern of dopamine release after systemic 6-AM is very different not only from that of heroin but also from that of morphine. On the one hand, the differences in the ability to increase dopaminergic transmission, which is thought to play a major role in motivation (Berke 2018), might be invoked to explain the differences in the reinforcing effects of 6-AM relative to heroin. On the other hand, it is not easy to explain the mechanisms responsible for these differences if the mechanisms of actions of heroin and of its metabolites are the same. Thus, it is possible that the intrinsic reinforcing effects of heroin, 6-AM, morphine, and M6G are mediated at least in part by distinct substrates. For example, since there is evidence that heroin self-administration is largely independent of striatal dopamine release (for a review, see Badiani et al., 2011), it would be important to determine if the same is true for 6-AM.

5. CONCLUSION

The present study shows that the heroin metabolite 6-AM is able to sustain self-administration behavior in the rat at a higher rate than equimolar doses of heroin. However, contrary to our working hypothesis, a 6-AM specific monoclonal antibody failed to affect heroin-induced relapse into drug-seeking and the re-acquisition of heroin self-administration after a period of relapse. It is possible that heroin deacetylation in the brain yielded sufficient 6-AM (out of the reach of mAb) to sustain self-administration. These findings indicate that more studies are required to clarify if and to what extent the reinforcing effects of heroin depend, as commonly held, on its metabolites.

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Figure 1. Time line of the experimental protocol used through the self-administration study.

Figure 2. Number (mean ±s.e.m.) of lever presses (upper panel) at the active (circles) or inactive (triangles) lever and infusions (lower panel, circles) during the acquisition and extinction of self-administration of 0.135 µmol/kg 6-AM (filled symbols) or heroin (open symbols). A FR1 schedule was used during the first five sessions of acquisition, and a FR2 schedule for the rest of the experiment (see Material and Methods for further detail).

Figure 3. Number (mean ± s.e.m.) of active lever presses (upper panel) and infusions (lower panel) during the first hour of the last extinction session (black columns) and the relapse session (grey columns). After the last extinction session, animals were treated ip with saline or 10 mg/kg of a monoclonal antibody against 6-AM (6-AM mAb). At the start of the relapse session, the animals were primed with a single infusion of 0.068 µmol/kg 6-AM or heroin. *$p<0.05$ versus last extinction session (post-hoc paired Student's t-tests).

Figure 4. Number (mean ± s.e.m.) of active lever presses (upper panel) and infusions (lower panel) during the last acquisition session, last extinction session, and the first re-acquisition session. After the last extinction session, animals were administered ip either with saline or 10 mg/kg of a monoclonal antibody against 6-AM (6-AM mAb). During the re-acquisition session, the animals self-administered 0.068 or 0.135 µmol/kg 6-AM or heroin. Whereas the columns for last day of acquisition and extinction represent data for all the animals together, two columns are used to represent data from animals pretreated with saline or 6-AM antibody. *$p<0.05$ versus Last Day Extinction (post-hoc paired Student's t-tests); # $p<0.05$ versus Last Day Acquisition (post-hoc paired Student's t-tests); ¤ $p<0.05$ versus 0.068 µmol/kg (post-hoc independent Student's t-tests); & $p<0.05$ versus re-acquisition Saline (post-hoc independent Student's t-tests).

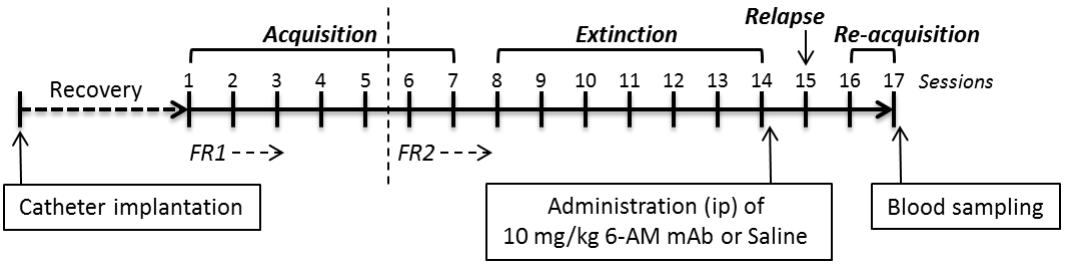
Acquisition
Extinction
Relapse
Re-acquisition
Recovery
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17
Sessions
FR1
FR2
Catheter implantation
Administration (ip) of
10 mg/kg 6-AM mAb or Saline
Blood sampling

Figure 1

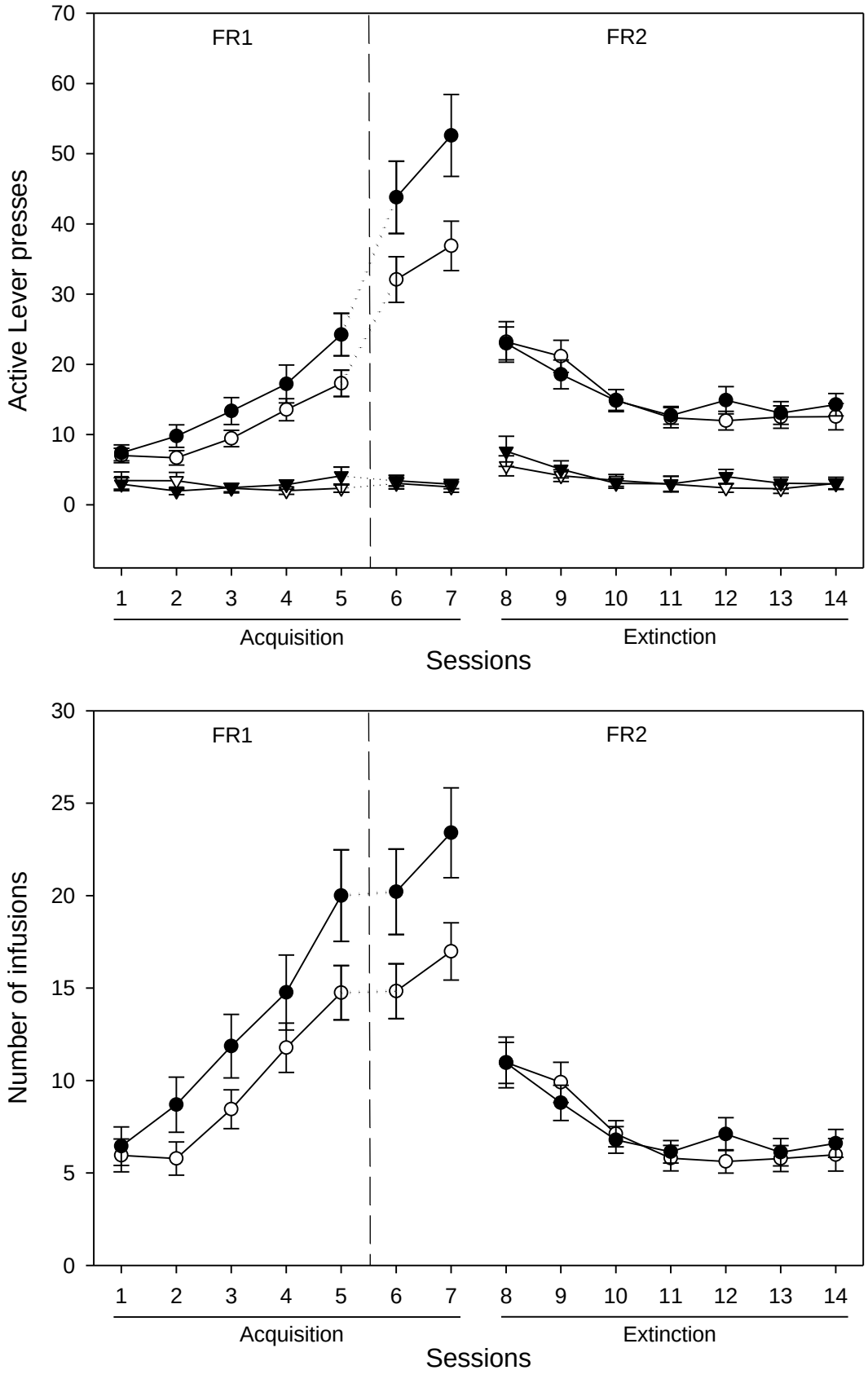
FR1
FR2
Active Lever presses
Number of infusions
Acquisition
Extinction
Sessions

Figure 2

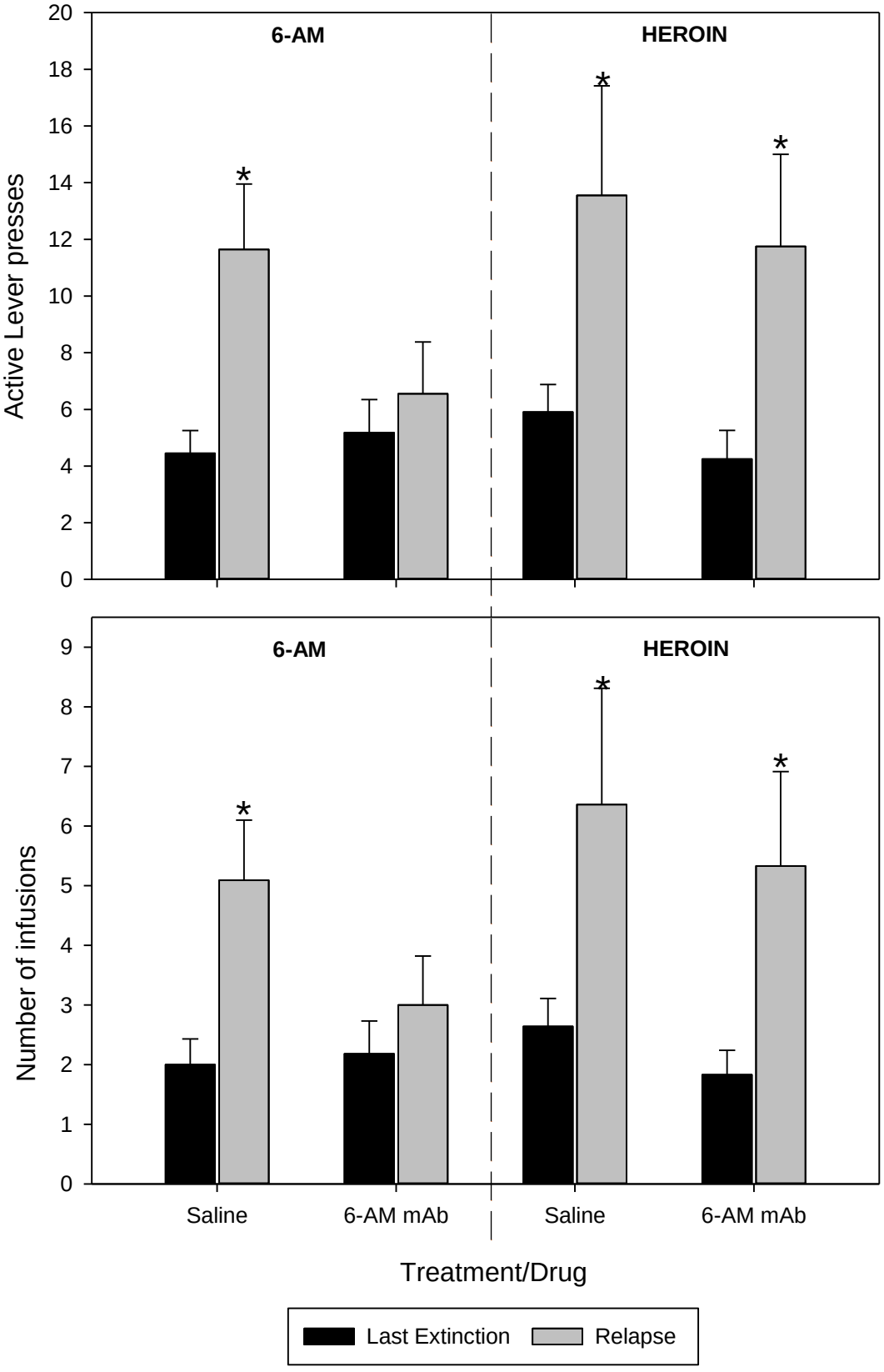
6-AM
HEROIN
Active Lever presses
20
18
16
14
12
10
8
6
4
2
0
6-AM
HEROIN
Number of infusions
9
8
7
6
5
4
3
2
1
0
Saline
6-AM mAb
Saline
6-AM mAb
Treatment/Drug
Last Extinction
Relapse

Figure 3

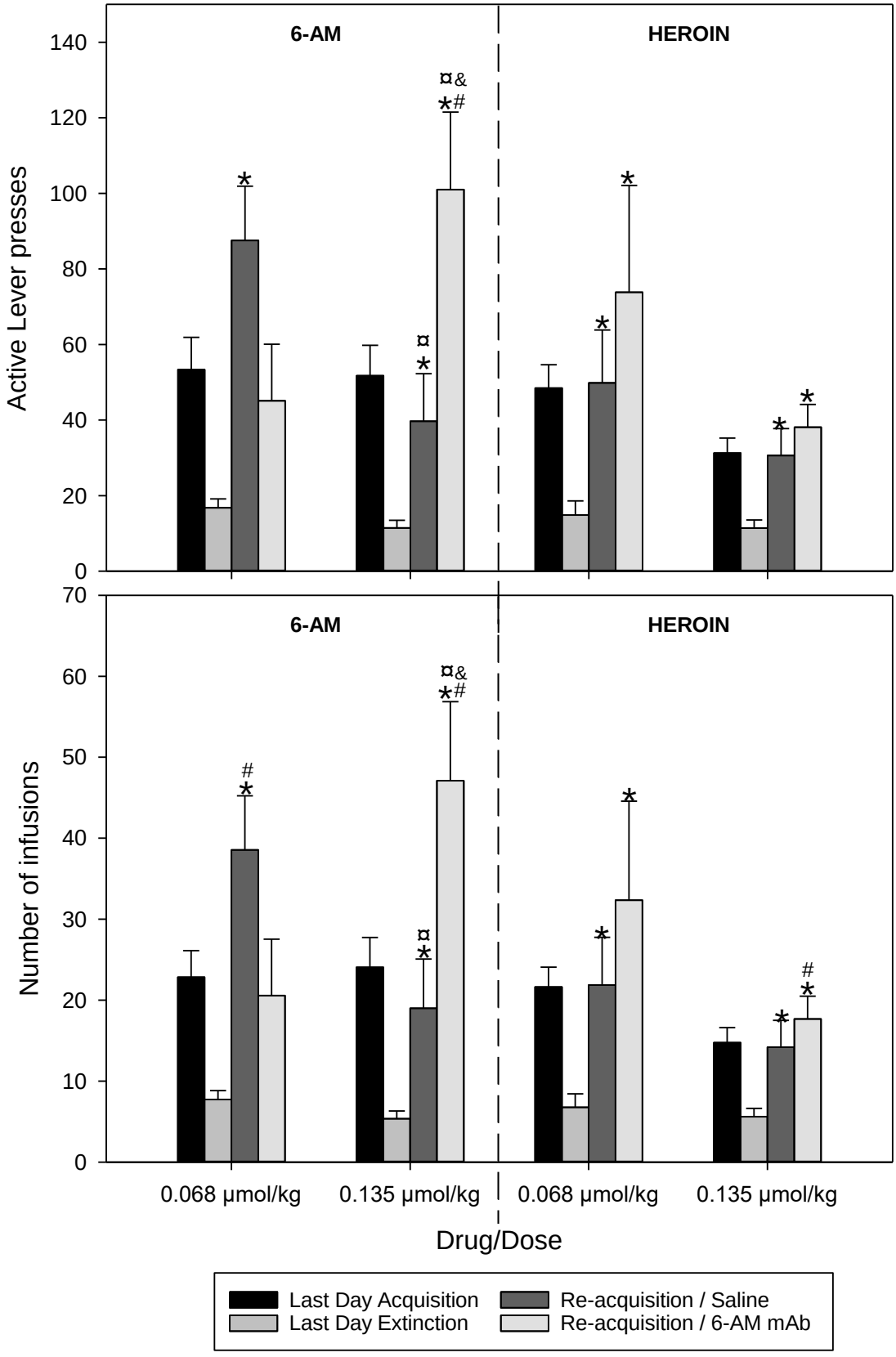
6-AM
HEROIN
Active Lever presses
Number of infusions
0.068 µmol/kg
0.135 µmol/kg
0.068 µmol/kg
0.135 µmol/kg
Drug/Dose
Last Day Acquisition
Last Day Extinction
Re-acquisition / Saline
Re-acquisition / 6-AM mAb

Figure 4